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Phytochemical study of *Harrisia adscendens*

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ABSTRACT

Harrisia adscendens (Gürke) Britton & Rose is a species of the family Cactaceae found in the northeastern semi-arid and popularly known as foxtail. In folk medicine, the roots of this species are used for the treatment of toothache and heartburn. The objective of this study was to perform the isolation and identification of the secondary metabolites obtained from the vegetal drug by chromatographic and spectroscopic techniques and to evaluate the antimicrobial activity of the extract. The qualitative phytochemical analysis of the extract showed suggestive results for the presence of alkaloids. Two compounds were isolated and identified: 2-methyl-9H-β-carboline-2-ion, a β-carboline alkaloid obtained for the first time as a natural product and 2',6'-dihydroxy-4'-methoxyacetophenone-2'-O-β-glucoside. In the antimicrobial tests, it was possible to observe activity against *Pseudomonas aeruginosa*. The results obtained by spectroscopic techniques allowed to characterize the phytochemical properties of the vegetal drug and may be useful in future studies for production of herbal medicines.

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Introduction

The World Health Organization (WHO, 2011) estimates that 70–90% of the population in developing countries need traditional medicine in primary health care. The culture of the use of plants with therapeutic properties has been valued by the society of the whole world.

Brazil has great potential for the development of phytotherapies/phytopharmaceuticals, since it presents the greatest plant diversity in the world. The use of these plants, linked to traditional knowledge and technology, can lead to the development of new drugs. About 82% of the Brazilian population uses herbal products as a therapeutic resource, from the consumption of the fresh plant and its derivatives to the use of herbal medicines (Ministério da Saúde, 2015).

Among the various families of plants found in Brazilian flora, the cacti stand out and call attention for their rusticity and beauty. They have great potential as a source of medicinal, cosmetic and food substances (Mariath et al., 2009), however phytochemical and pharmacological studies with these plants are scarce, although it is known that many species of the Cactaceae family have medicinal

properties in the treatment of ulcers and bronchitis (Davet et al., 2009), as well as anti-inflammatory, diuretic and antiglycemic activity (Necchi et al., 2012; Dias et al., 2015).

The Cactaceae family is composed of approximately 1405 species divided into 127 genera (Barthlott and Hunt, 1993; Areces, 2004; Nyffeler and Egli, 2010; Brito-Filho et al., 2017). They present the subfamily Cactoideae and are divided in three tribes – Pereskieae, Opuntieae and Cereae – being that *Harrisia adscendens* belongs to the Cereae tribe (Barroso et al., 1978). *Harrisia adscendens* (Gürke) Britton and Rose is a native caatinga (savana) species, popularly known as foxtail and is used in traditional medicine for the treatment of “burning”, kidney problems and toothaches (Albuquerque et al., 2007).

Based on the above mentioned and in the perspective of collaborating for new therapeutic alternatives and confirming the popular information, the purpose of this work was to perform a phytochemical and microbiological screening of the ethanolic extracts and hydroalcoholics, as well as to isolate and identify possible substances originating from the secondary metabolism of the plant species through chromatographic and spectroscopic techniques.

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Material and methods

Plant material

The roots were collected in March 2014, in the rural area of the city of Itaporanga, Paraíba backwoods (latitude 7,1845 S and longitude 38,0959 O). An exsiccata was prepared and sent to the Herbarium Professor Lauro Pires Xavier of the Federal University of Paraíba, for proper identification and botanical name, registered under the number JPB 61259.

After harvesting, the roots were dehydrated in an air circulating oven, under a temperature of 40 °C for 96 h and then crushed in a mechanical knife mill, obtaining 1.430 g of the vegetal drug.

General experimental procedures

Chromatographic columns were packed with neutral aluminum oxide (70–270 mesh ASTM, Macherey-Nagel) and silica gel 60 (70–270 mesh ASTM, Macherey-Nagel) using glass columns and medium-pressure liquid chromatography (MPLC-model BÜCHI 688). Thin-layer chromatography (TLC) was performed on PF₂₅₄ plates, and the spots were visualized under ultraviolet light (254 and 365 nm) and by Dragendorff's reagents. Isolated compounds were identified by infrared (IR; Perkin-Elmer, FT-IR-1750, and Shimadzu, IR prestige 21); the mass spectra was obtained using MS analysis performed on an Amazon X (Bruker) and high-resolution on a microTOF-II model (Bruker); and extensive one- and two-dimensional nuclear magnetic resonance (NMR) analysis acquired on the following spectrometers: 500 MHz Varian Mercury – IpeFarM/UFPB; 500 MHz Bruker-AC, CENAUREMN/UFC using deuterated solvents.

Extraction and isolation

The plant drug (1250 g) was treated with EtOH (6 × 21) with a 72 h interval to maximize the extraction process. The ethanolic extract was concentrated on a rotary evaporator to give 125 g of the crude ethanolic extract (CEE). Five samples (25 g each) of the plant drug were subjected to maceration with ethanol:water (v:v) solutions (125 ml) in the following proportions: 9:1, 7:3, 1:1, 3:7 and 1:9 to obtain the crude hydroalcoholic extracts (CHE). These macerates were also concentrated in a rotary evaporator and subjected to hot air flow for the evaporation of the solvents.

The CEE obtained underwent a march for the extraction of alkaloids and was treated with 2l of a 3% HCl acid solution and subsequently filtered on filter paper. The residue was discarded and the filtrate was subjected to several extractions with chloroform. The acidic aqueous phase was basified with NH₄OH, under vigorous stirring, to pH 10 and extracted with chloroform. Subsequently, the chloroform fraction was evaporated under reduced pressure in a rotary evaporator at 40 °C yielding 1.95 g of the total alkaloid fraction (TAF).

The TAF (1.2 g) was chromatographed over an aluminum oxide (36 g) and eluted using a gradient of solvents with increasing polarity (dichloromethane, ethyl acetate and methanol). From this process, 66 fractions were obtained and combined by TLC. Compound **1** was the yellow crystal (12 mg) from fractions 46–47. Others 750 mg of the TAF were chromatographed on a silica gel column (22.5 g) under medium-pressure liquid chromatography, using hexane, ethyl acetate and methanol pure or in binary mixture and yielding 54 fractions. The pure fractions 14–15, eluted in ethyl acetate and methanol (9:1), yielded an amorphous white solid (10 mg) identified as the compound **2**.

Antimicrobial activity assay

Microorganisms, microbial inoculum and preparation of CEE and CHE solutions

The microbial strains used in this study came from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 76645 and *Candida tropicalis* ATCC 1369. The selected strains were seeded in BHI broth for reactivation and incubated for 24 h/37 °C. After reactivation, the microorganisms were cultured in Mueller Hinton Agar for 18 h/37 °C, isolated colonies were transferred to sterile saline (0.85%) in turbidity equivalent to 0.5 McFarland scale (10⁸ CFU ml⁻¹) (CLSI, 2016). CEE and CHE were solubilized in dimethylsulfoxide to 2% (aqueous DMSO) at an initial concentration of 8000 µg ml⁻¹.

Determination of minimum inhibitory concentration (MIC)

The MIC determination of CEE and CHE was performed by the microplate microdilution test (CLSI, 2016). Sterile 96-well microplates with lid were used in which 100 µl of Mueller Hinton broth were distributed aseptically. In the first well of each microplate, 100 µl of the test product (one for CEE and one for each CHE) at a concentration of 8000 µg ml⁻¹ was added. Dilutions of 4000 to 300 µg ml⁻¹ were performed. After the dilutions, 5 µl of the microbial inoculum was added to the horizontal lines (numbered 1–12). Each of the numbered lines corresponded to one strain. The positive control of the microbial growth was constituted by means of culture and 5 µl of the inoculum, the negative control only with the culture medium. It was used also gentamicin as control of antimicrobial activity against bacterial strains and fluconazole against *Candida* strains, besides the negative control of DMSO to 2%. The plates were closed, sealed and incubated for 24 h/37 °C. After the incubation period, 20 µl of 0.01% aqueous resazurin was added to each well. The plates were kept at room temperature for 2 h elutes after that time. The reading was performed visually characterized by the color change in the cavities. Results were read by viewing the color change from blue to pink for bacteria, and from colorless to pink for yeast, indicating growth of the microorganism. The lowest concentration of the product capable of inhibiting the growth of the microbial strain tested was considered as MIC (Mann and Markham, 1998; Salvat et al., 2001; Burt and Reinders, 2003; Alves, 2006). The tests were performed in triplicate and the result expressed by the mean.

Results and discussion

The structural assignments of compounds **1** and **2** were made based on spectral analysis and are in good agreement with those reported in the literature. Analysis of the mass spectrum of **1** gave a molecular formula C₁₂H₁₁N₂⁺ determined by ESI/MS with *m/z* 183.09 confirmed by the high resolution mass spectrum obtained by microTOF-II, which showed a molecular ion peak in *m/z* 183.0933 (calculated for C₁₂H₁₁N₂⁺, 183.2340) and a peak for the base ion in *m/z* 168.0703 suggestive of the outflow of a methyl group. In the ¹H NMR spectrum (δ, DMSO-d₆, 500 MHz) eight signals were visualized, seven of which were characteristic of hydrogens in aromatic systems at δ_H 9.27 (s, 1H), 8.70 (d, *J* = 6.2 Hz, 1H), 8.43 (d, *J* = 8.3 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H) indicative of the presence of a 1,2-disubstituted phenyl ring which together with (δ_H 8.51 and δ_H 8.70) characterize α,β pyridine protons suggesting that the molecule is a β-carboline alkaloid (Gearhart et al., 2002), a coalescing multiplet of δ_H 7.76–7.81 (2H), which was split into two signals [7.78 (d, *J* = 8.3 Hz, 1H) and 7.74 (t, *J* = 7.4 Hz, 1H)] when the temperature of the experiment was increased from 26 to 60 °C, in addition to a singlet δ_H 4.47 (3H) which in HSQC (¹H × ¹³C)

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